REMARKS

Claims 1-53 were pending before this Response. By the present communication, Abstract and paragraph numbers 0147, 0148, 0185, 0189 and 0194 (mistakenly cited as 0197) in the Specification have been amended to overcome informalities as shown in attached Exhibit A. In addition, claims 1, 2, 5, 8, 10, 13,15, 19, 20, 26, 27, 28, 31, 34, 36, 39, 41, and 46 have been amended and new claims 54-55 have been added as shown in attached Exhibit A. The amendments add no new matter, being fully supported by the Specification and original claims. Accordingly claims 1-55 are currently pending.

The Objection to the Specification

The disclosure is objected to for allegedly containing informalities. To overcome the objection to the disclosure, the following amendments have been made to the Specification.

In the Abstract, the spelling of the word "nucleic" has been corrected.

The Examiner asserts that Paragraph [0147] beginning on page 41 requires correction by corrected by substitution of the symbol "ß" for "(" at four locations. Applicants have amended Paragraph [0147] by substitution of the symbol "ß" for "(" at six locations.

The Examiner asserts that Paragraph [0148] beginning on page 42 requires correction by by substitution of the symbol "ß" for "(" at three locations. Applicants have amended Paragraph [0148] by substitution of the symbol "ß" for "(" at three locations.

Paragraph [0185] beginning on page 54 has been corrected by deletion of the sentence that refers to "Figure X" and correction of the typographical error in the phrase "which can the be decorated" to read instead: "which can then be decorated".

Paragraph [0189] beginning on page 56 has been corrected by substitution of the word "growth" for "grown".

Paragraph [0194] beginning on page 57 (mistakenly described by the Examiner as paragraph [0197]) has been corrected by deletion of "can" from the incorrect phrase "compounds can are utilized".

Applicants respectfully submit that the above-described amendments to the Specification overcome the grounds for the objection to the Specification and reconsideration and withdrawal are respectfully requested.

The Objection to the Drawings

The Office Action indicates the drawings are objected to on various grounds. Applicants submit new corrected Figures to overcome the objection to the drawings as follows: In Figure 7, the misspelling of "fluor" has been corrected. In Figure 14, "from host" has been replaced by "the library"; and in Figure 15, the misspelling of "growth" has been corrected. Applicants respectfully submit that corrected Figures 7, 14 and 15 submitted herewith overcome the grounds for the objection to the drawings and reconsideration and withdrawal are respectfully requested.

The Objection to the Claims

Claims 2, 10, 13, 15, 20, 27, 28 36, 39, 41 and 46 have been objected to for allegedly containing informalities. The following amendments have been entered to overcome the objection to the claims.

In claims 2 and 28, the word "is" has been deleted from the phrase "provided by an enzyme is selected from" to conform to the usual terminology for claiming alternatives.

In claims 10 and 36, the misspelling of the term "extremophiles" has been corrected.

In claims 13 and 39, duplication of the word "the" has been corrected.

In claims 15 and 41, the phrase "occurs at about 30 minutes" has been amended to read "occurs for about 30 minutes".

In claims 20 and 46, for clarity the term "library in" has been added so as to read "is transferred to a library in *Streptomyces* sp."

In claim 27, the word "encapsulating" has been substituted for the term "encapsulation" to indicate an active method step.

With regard to claim 31, the Examiner asserts that there is insufficient antecedent basis for the term "the Streptomyces." To clarify the antecedent basis for this term, claim 31 has been amended to insert "sp." after "Streptomyces", thus clarifying antecedent basis in claim 30, from which claim 31 depends.

With regard to claim 34, the Examiner asserts that there is insufficient antecedent basis for the term "the expression library." To clarify the antecedent basis for this term, claim 34 has been amended to change dependency to claim 33, the term "an expression library" in claim 33 thus providing antecedent basis for the term "the expression library" in claim 34.

Applicants respectfully submit that the above amendments overcome all informalities in the present claims, and reconsideration and withdrawal of the rejection for informalities in the claims are respectfully requested.

The Rejection under 35 U.S.C. § 112, Second Paragraph

Applicants respectfully traverse the rejection of claims 1-53 under 35 U.S.C. § 112, Second Paragraph, as allegedly being indefinite. With regard to alleged indefiniteness of claim 1, the Examiner asserts that inconsistent terminology (i.e. use of both "change" and "difference") renders the metes and bounds of the claim uncertain. To provide internal consistency of terminology, claim 1 has been amended to substitute "difference" for "change". The Examiner further asserts that claim 1 is vague and indefinite due to alleged failure to include a method step that "clearly relates back to the preamble" (Office Action, page 4). To overcome this alleged source of indefiniteness, the final step of claim 1 has been amended to recite: "screening the microdroplets with an assay or an analyzer that detects the presence therein of the change in the substrate, wherein the change in the substrate indicates the identity of the bioactivity or biomolecule", thus matching the language of the preamble with that of the final method step. In addition, an amendment has been made in line 3 of claim 1 to delete "the" before "DNA" to remove any lack of clarity regarding antecedent basis for the term "DNA".

With regard to claim 5, the examiner asserts that there is insufficient antecedent basis for the term "the Streptomyces." To clarify the antecedent basis for this term, claim 5 has been amended to insert "sp." after "Streptomyces", thus clarifying antecedent basis in claim 4, from which claim 5 depends.

With regard to claim 8, the examiner asserts that there is insufficient antecedent basis for the term "the expression library." To clarify the antecedent basis for this term, claim 8 has been amended to change dependency to claim 7, the term "an expression library" in claim 7 thus providing antecedent basis for the term "the expression library" in claim 8.

With regard to claim 19, the examiner asserts that there is insufficient antecedent basis for the term "the prokaryotic cell." To clarify the antecedent basis for this term, claim 19 has been amended to change dependency to claim 3, the term "a prokaryotic cell" in claim 3 thus providing antecedent basis for the term "the prokaryotic cell" in claim 19.

The Examiner further asserts that claim 26 is vague and indefinite due to alleged failure to include a method step that "clearly relates back to the preamble" (Office Action, page 5). To overcome this alleged source of indefiniteness, the final method step of claim 26 has been amended to recite: "screening the clones with an assay or an analyzer that detects the presence therein of the change in the substrate, wherein the change in the substrate identifies the bioactivity or biomolecule", thus matching the language of the preamble with that of the final method step.

With regard to claim 31, the Examiner asserts that there is insufficient antecedent basis for the term "the Streptomyces." To clarify the antecedent basis for this term, claim 31 has been amended to insert "sp." after "Streptomyces", thus clarifying antecedent basis in claim 30, from which claim 31 depends.

With regard to claim 34, the Examiner asserts that there is insufficient antecedent basis for the term "the expression library." To clarify the antecedent basis for this term, claim 34 has been amended to change dependency to claim 33, the term "an expression library" in claim 33 thus providing antecedent basis for the term "the expression library" in claim 34.

With regard to claims 13 and 39, the Examiner asserts that there is insufficient antecedent basis for the limitation "the samples." To overcome the alleged lack of antecedent basis, claims 13 and 39 have been amended to delete "the the", thus no antecedent is implied for "samples" and any lack of clarity regarding antecedent basis for the term "samples" in claims 13 and 39 is overcome.

In view of the above amendments, Applicants submit that the invention as presently claimed meets all requirements under 35 U.S.C. § 112, Second Paragraph, and reconsideration and withdrawal of the rejection are respectfully requested.

The Double Patenting Rejections

Applicants respectfully traverse the rejection of claims 16, 17, 42 and 43 under 35 U.S.C. § 101 as allegedly claiming the "same invention" as that of claims 11 and 42 of prior U.S. Patent No. 6, 174,673 (hereinafter "'673"). The Examiner acknowledges that for a prior U.S. patent to be used in a rejection for statutory double patenting, the prior U.S. patent must have an invention drawn to "identical subject matter" (Office Action, page 6). A reliable test for double patenting under 35 U.S.C. § 101 is whether a claim in the application could be literally infringed without literally infringing a corresponding claim in the patent. *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970). Is there an embodiment of the invention that falls within the scope of one claim, but not the other? If there is such an embodiment, then identical subject matter is not defined by both claims and statutory double patenting would not exist. M.P.E.P. § 804.

Applicants respectfully disagree that the subject matter of Applicants' claims 16, 17, 42 and 43 is "identical" to that of claims 11 and 42 of '673. Further, Applicants would like to

respectfully call to the attention of the Examiner that the '673 reference does not contain 42 claims. Applicants acknowledge that '673 contains two independent claims (1 and 22), and therefore assume that Examiner meant to cite claims 11 and 22 of '673.

Present claims 16, 17, 42 and 43 are all dependent claims and thus include the subject matter of amended claims 1 or 26, from which they depend. Applicants respectfully submit that the invention methods for identifying bioactivities or biomolecules using high through-put screening, as defined by amended claim 1, distinguish over the subject matter of claims 11 and 22 of '673 by requiring "providing a gene library containing a plurality of clones, wherein DNA (or nucleic acid) for generating the library is obtained from more than one organism; encapsulating a bioactive substrate and at least one clone of the library in a gel microdroplet, wherein a bioactivity or biomolecule produced by the clone is detectable by a difference in the substrate prior to contacting with the at least one clone as compared to after the contacting; and screening the microdroplets with an assay or an analyzer that detects the presence therein of the change in the substrate, wherein the change in the substrate identifies the bioactivity or biomolecule." Similarly, the claim language of claim 26 distinguishes over that of claims 11 and 22 of '673 by requiring "providing a gene library containing a plurality of clones, wherein the nucleic acid for generating the library is obtained from more than one organism, inserting a bioactive substrate into the clones of the library, wherein a change in the substrate is detectable in the presence of a bioactivity or biomolecule; and screening the clones with an assay or an analyzer that detects the presence therein of the change in the substrate, wherein the change in the substrate identifies the bioactivity or biomolecule.

By contrast, claims 11 and 22 of '673 do not recite subject matter that is identical to claims 1 and 26 of the invention, i.e., an identical method for identifying bioactivities or biomolecules using high throughput screening, for example one wherein screening of the microdroplets or of clones containing an inserted substrate identifies "the identity of the bioactivity or biomolecule" itself rather than simply identifying a clone that contains DNA encoding a bioactivity or biomolecule. Further, the claim language of both claims 11 and 22 differ by requiring "providing an expression library containing a plurality of clones...." Thus, there is an embodiment of the invention that falls within the scope of one claim, but not the other. Therefore, Applicants respectfully submit that the test for establishing statutory double patenting under 35 U.S.C. § 101 is not met by any claim of U.S. Patent 6,174,673. Consequently, reconsideration and withdrawal of the rejection thereunder are respectfully requested.

Applicants also respectfully traverse the rejection of claims 1-15, 18-41 and 44-53 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-23 of '673. Applicants submit herewith a Terminal Disclaimer disclaiming the terminal part of any patent granted on the above-identified Application No. 09/848,095 that would extend beyond the expiration date of U. S. Patent No. 6,174,673, which shall be enforceable only so long as the legal title to such patent shall be the same as the legal title to U.S. Patent No. 6,174,673. Further, Applicants declare that the subject matter of the above-identified Application No. 09/848,095 and U.S. Patent 6,174,673 were co-owned by Diversa Corporation at the filing date of this application.

In view of the Terminal Disclaimer submitted herewith, Applicants submit that U.S. Patent 6,174,673 is not a reference against present claims 1-15, 18-41 and 44-53 under the doctrine of obviousness type double patenting, and reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection under 35 U.S.C. § 102(e)

Applicants respectfully traverse the rejection of claims 1-4, 6-10, 18, 19, 23-30, 32-36, 39-41, 44, 45 and 51-53 under 35 U.S.C. § 102(e) as allegedly being anticipated by U.S. Patent No. 5,824,485 (hereinafter "Thompson"). The invention methods for identifying bioactivities or biomolecules using high throughput screening of nucleic acid, as defined by amended claim 1, distinguish over the disclosure of Thompson by requiring "providing a gene library containing a plurality of clones, wherein DNA for generating the library is obtained from more than one organism, encapsulating a bioactive substrate and at least one clone of the library in a microdroplet, wherein a bioactivity or biomolecule produced by the clone is detectable by a change in the substrate prior to contacting with the at least one clone as compared to after the contacting, and screening the microdroplet with an assay or an analyzer that detects the presence therein of the change in the substrate, wherein the change indicates the identity of the bioactivity or biomolecule." In the method of claim 26, the bioactive substrate is inserted directly into the clone and the clone is screened to determine a change in the substrate that is indicative of the presence and identity of the biomolecule or bioactivity, i.e., a particular biomolecule or bioactivity.

Thompson fails to disclose each and every aspect of the invention methods as defined by amended claims 1 and 26. For example, Thompson fails to disclose co-encapsulation in a gel microdroplet of a library clone and a detectable bioactive substrate wherein the bioactive substrate comprises a substrate for the desired biomolecule or bioactivity of interest and a change in the substrate, such as a change in fluorescence, indicates the presence and identity of the bioactivity or biomolecule that can be detected by screening of the microdroplet. Similarly, Thompson fails to disclose insertion into a library clone of such a bioactive substrate or of a polynucleotide encoding such a substrate. Instead, when Thompson refers to a "substrate", the term is used to describe substrates "supplied by the host organism, by other heterologous gene products that are co-expressing in the same host organism, or from the medium" (Thompson, Col 30, lines 2-5). Elsewhere Thompson describes "fluorescent probes" as substrates, but the disclosed probes are non-specific, reflecting general "metabolic activities" such as "decrease in membrane potential, intracellular pH or increase in cytochromes-mediated oxidation" (See Thompson, Col 37, lines 10-25). Thus, Applicants respectfully submit that Thompson fails to disclose each and every aspect of the invention methods for identifying bioactivities or biomolecules using high throughput screening of nucleic acids, as would be required to support a rejection for anticipation under 35 U.S.C. § 102(e). Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection for alleged anticipation of claims 1-5 and 8-18, 20 and 24-26 over the disclosure of Thompson.

The Rejection Under 35 U.S.C. § 103

Applicants respectfully traverse the rejection of claims 1-4, 6-15, 18, 19, 23-30, 32-41, 44, 45 and 51-53 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Thompson as applied to claims 1-4, 6-10, 18, 19, 23-30, 32-36, 39-41, 44, 45 and 51-53 in the rejection under 35 U.S.C. § 102 above and further in view of Plovins et al. (App. Environ. Microbiology (1994) 60:4638-4641; hereinafter "Plovins") and Zhang et al. (FASEB J. (1991) 5:3108-3113; hereinafter "Zhang"). To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990)

Applicants respectfully submit that the invention methods for identifying bioactivities or biomolecules using high throughput screening of nucleic acid, as defined by amended claims 1 and 26, distinguish over the combined disclosures of Thompson, Plovins and Zhang for the same reasons as discussed above with reference to Thompson. For example, Thompson fails to disclose co-encapsulation in a microdroplet of a library clone and a detectable bioactive substrate

wherein the bioactive substrate comprises a substrate for the desired biomolecule or bioactivity and a change in the substrate, such as a change in fluorescence, as indicated by screening of the microdroplet, indicates the presence and identity of the bioactivity or biomolecule. Similarly, Thompson fails to disclose insertion into the clone of such a bioactive substrate, such that the presence of the clone causes a change in the substrate, such as a change in fluorescence. Thus, screening of the clones indicates both the presence and the identity of the bioactivity or biomolecule.

Plovins fails to cure these deficiencies in Thompson. The Examiner relies upon Plovins as disclosing use of FDG as well as C_{12}FDG as substrates in animal, bacterial and yeast cells. However, Plovins fails to disclose co-encapsulation of FDG or C_{12}FDG with a DNA in a gel microdroplet for screening of whole microdroplets. Similarly, Plovins fails to disclose insertion of a polynucleotide encoding a substrate directly into a library of DNA clones wherein a bioactivity or biomolecule produced by the clone changes the substrate, indicating the presence and identity of the bioactivity or biomolecule in the clone. Thus, Applicants respectfully submit that the combined disclosures of Thompson and Plovins fail to teach or suggest the invention methods for identifying bioactivities or biomolecules using high throughput screening, as defined by amended claims 1 and 26.

Like Plovins, Zhang also fails to cure the deficiencies of Thompson for teaching or suggesting the invention methods for identifying bioactivities or biomolecules using high throughput screening, as defined by amended claims 1 and 26. The Examiner relies upon Zhang for disclosure of the development of lipophilic, fluorogenic substrates derived from FDG, such as FDG having an added lipophilic tail, to enable the substrate to pass through the cellular membrane. However, Zhang fails to disclose co-encapsulation of such a substrate with library

DNA in a gel microdroplet for detection of microdroplets wherein a change in the substrate signals the presence and identity of a clone that interacts with the substrate to cause the change. Similarly, Zhang fails to disclose or suggest insertion into a library clone of a bioactive substrate for identification of clones that cause a change in the substrate, thereby identifying the identity of the substrate. In view of the failure of either Plovins or Zhang to cure the above-described deficiencies of Thompson for suggesting the invention methods, Applicants respectfully submit that the combined disclosures of Thompson, Plovins and Zhang are not sufficient to teach or suggest the present invention, as defined by amended claims 1 and 26. Further, even if the Examiner does not find the above argument persuasive, Applicant respectfully asserts that absent the present disclosure, the Examiner has not provided a reference that teaches or suggests the combination of the prior art references.

Applicants also respectfully traverse the rejection of claims 1-10, 18, 19, 20-30, 31-36, 39-41, 44 and 46-53 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Thompson as applied to claims 1-4, 6-10, 18, 19, 23-30, 32-36, 39-41, 44, 45 and 51-53 in the rejection under 35 U.S.C. § 102 above and further in view of Short (U.S. Patent No. 6,057,103; hereinafter "Short"). In order to show that the prior art suggests the invention in question, the Examiner has the burden of establishing a prima facie case by satisfying three requirements: a) the prior art relied upon, coupled with the knowledge generally available in the art at the time of the invention, must contain some suggestion or incentive that would have motivated those skilled in the art to modify a reference or to combine references; b) the proposed modification of the prior art must have had a reasonable expectation of success, determined from the vantage point of those skilled in the art at the time the invention was made; c) the prior art reference or

combination of references must teach or suggest all the limitations of the claims, and the teachings or suggestions, as well as the expectation of success, must come from the prior art, not the applicant's disclosure (can't use the disclosure as a blueprint to reconstruct the claimed invention from isolated pieces of the prior art).

The deficiencies of Thompson for disclosing the invention methods for identifying bioactivities or biomolecules using high throughput screening of nucleic acid discussed above apply equally here. In particular, Thompson fails to disclose co-encapsulation in a gel microdroplet of a library clone and a detectable bioactive substrate wherein the bioactive substrate comprises a substrate for the desired biomolecule or bioactivity of interest and a change in the substrate, such as a change in fluorescence, wherein screening of the microdroplets detects the change, indicating the presence and identity of the bioactivity or biomolecule. Similarly, Thompson fails to disclose insertion directly into a library clone of such a bioactive substrate, and screening of the clones to determine a change in the substrate, such change indicating the presence and identity of the biomolecule or bioactivity of interest in the clone. Applicants respectfully submit that Short fails to cure these deficiencies of Thompson for teaching or suggesting the present invention. Rather, the Examiner relies upon Short for disclosing transfer of a library from *E. coli* to *Streptomyces* prior to encapsulation and screening or a normalization step prior to encapsulation and screening (Office Action, page 11). Thus, Applicant respectfully submits that Short does not provide those of skill in the art with any motivation to modify Thompson along the lines of Applicants' invention. Moreover, the Examiner has failed to establish that the general knowledge in the art at the time of the invention was such that those of skill in the art would have been motivated to combine the Thompson and Short references.

In addition, Applicants submit that the Examiner has provided no reason to suppose that those of skill in the art would have had a reasonable expectation that modification of Thompson's disclosure along the lines of the present invention would be successful in view of the combined disclosures of Thompson and Short.

Therefore, Applicants respectfully submit that the combination of the references relied upon in this rejection as well as any expectation of success imputed by the Examiner to those of skill in the art at the time the application was filed for modifying the combined references is based upon impermissible hindsight provided by Applicants' own disclosure. Thus, Applicants submit that the Examiner has used Applicants' specification with hindsight as a blueprint to reconstruct the claimed invention from Thompson and Short.

Therefore, in view of the above amendments and remarks, Applicants respectfully submit that *prima facie* obviousness of the invention methods, as defined by present claims 1-55, is not shown by the combined disclosures of Thompson, Zhang and Plovins or by the combined disclosures of Thompson and Short and reconsideration and withdrawal of the rejections under 35 U.S.C. § 103(a) are respectfully requested.

In view of the Terminal Disclaimer submitted herewith and the above amendments and remarks, Applicants respectfully request allowance of claims 1-55. If the Examiner would like to discuss any of the issues raised in the Office Action, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

Date: August 20, 2002

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Enclosures: Exhibit A
Terminal Disclaimer
Corrected drawings

Applicants: Short and Keller
Application No.: 09/848,095
Filed: May 3, 2001
Exhibit A: Page 1

EXHIBIT A

Version with Markings to Show Changes Made

Please amend the Abstract of the invention as follows:

(Amended) Disclosed is a process for identifying clones having a specified activity of interest, which process comprises (i) generating one or more expression libraries derived from [nuclei] nucleic acid directly isolated from the environment; and (ii) screening said libraries utilizing a fluorescence activated cell sorter to identify said clones. More particularly, this is a process for identifying clones having a specified activity of interest by (i) generating one or more expression libraries derived from nucleic acid directly or indirectly isolated from the environment; (ii) exposing said libraries to a particular substrate or substrates of interest; and (iii) screening said exposed libraries utilizing a fluorescence activated cell sorter to identify clones which react with the substrate or substrates. Also provided is a process for identifying clones having a specified activity of interest by (i) generating one or more expression libraries derived from nucleic acid directly or indirectly isolated from the environment; and (ii) screening said exposed libraries utilizing an assay requiring co-encapsulation, a binding event or the covalent modification of a target, and a fluorescence activated cell sorter to identify positive clones.

Please amend Paragraph [0147] beginning on page 41 to read as follows:

(Amended) Several methods have been described for using reporter genes to measure gene expression. These reporter genes encode enzymes not ordinarily found in the type of cell

being studied, and their unique activity is monitored to determine the degree of transcription. Nolan *et al.*, developed a technique to analyze [(]ß -galactosidase expression in mammalian cells employing fluorescein-di-[(]ß -D-galactopyranoside (FDG) as a substrate for [(]ß -galactosidase, which releases fluorescein, a product that can be detected by a fluorescence-activated cell sorter (FACS) upon hydrolysis (Nolan *et al.*, 1991). A problem with the use of FDG is that if the assay is performed at room temperature, the fluorescence leaks out of the positively stained cells. A similar problem was encountered in other studies of [(]ß -galactosidase measurements in mammalian cells and yeast with FDG as well as other substrates (Nolan *et al*, 1988; Wittrup *et al.*, 1988). Performing the reaction at 0°C appreciably decreased the extent of this leakage of fluorescence (Nolan *et al.*, 1988). However this low temperature is not adaptable for screening for, for instance, high temperature [(]ß -galactosidases. Other fluorogenic substrates have been developed, such as 5-dodecanoylamino fluorescein di-[(]ß -D-galactopyranoside (C[12-]$_{12}$-FDG) (Molecular Probes) which differs from FDG in that it is a lipophilic fluorescein derivative that can easily cross most cell membranes under physiological culture conditions. The green fluorescent enzymatic hydrolysis product is retained for hours to days in the membrane of those cells that actively express the *lacZ* reporter gene. In animal cells C[12-]$_{12}$-FDG was a much better substrate, giving a signal which was 100 times higher than the one obtained with FDG (Plovins et al., 1994). However in Gram negative bacteria like *E. coli,* the outer membrane functions as a barrier for the lipophilic molecule C[12-]$_{12}$-FDG and it only passes through this barrier if the cells are dead or damaged (Plovins et al). The fact that C_{12} retains FDG substrate inside the cells indicates that the addition of unpolarized tails may be used for retaining substrate inside the cells with respect to other enzyme substrates.

Applicants: Short and Keller
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Filed: May 3, 2001
Exhibit A: Page 3

Please amend Paragraph [0148] on page 42 to read as follows:

(Amended) The abovementioned [(]ß-galactosidase assays may be employed to screen single *E. coli* cells, expressing recombinant [(]ß -D-galactosidase isolated from a hyperthermophilic archaeon such as *Sulfolobus solfataricus*, on a fluorescent microscope. Cells are cultivated overnight, centrifuged and washed in deionized water and stained with FDG. To increase enzyme activity, cells are heated to 70°C for 30 minutes and examined with a fluorescence phase contrast microscope. *E. coli* cell suspensions of the [(]ß -galactosidase expressing clone stained with C[12-]12-FDG show a very bright fluorescence inside single cells ([Fig] Figure 8).

Please amend Paragraph [0185] on page 54 to read as follows:

(Amended) Probe nucleic acid sequences designed according to the method described above can also be utilized in the present invention to "enrich" a population for desirable clones. "Enrich", as utilized herein, means reducing the number and/or complexity of an original population of molecules. For example, probes are designed to identify specific polyketide sequences, and utilized to enrich for clones encoding polyketide pathways. [Figure X depicts in-situ hybridization of encapsulated fosmid clones with specific probes of interest, in this case polyketide synthase gene probes.] Fosmid libraries are generated in E.coli according to the methods described in the Example herein. Clones are encapsulated and grown to yield encapsulated clonal populations. Cells are lysed and neutralized, and exposed to the probe of interest. Hybridization yields a positive fluorescent signal which can be sorted on a fluorescent cell sorter. Positives can be further screened via expression, or activity, screening. Thus, this aspect of the present invention facilitates the reduction of the complexity of the original

population to enrich for desirable pathway clones. These clones can the be utilized for further downstream screening. For example, these clones can be expressed to yield backbone structures (defined herein), which can [the] then be decorated in metabolically rich hosts, and finally screened for an activity of interest. Alternatively, clones can be expressed to yield small molecules directly, which can be screened for an activity of interest. Further more, multiple probes can be designed and utilized to allow "multiplex" screening and/or enrichment. "Multiplex" screening and/or enrichment as used herein means that one is screening and/or enriching for more than desirable outcome, simultaneously.

Please amend Paragraph [0189] on page 56 to read as follows:

(Amended) *Streptomyces venezuelae*, unlike most other *Streptomyces* species, has been shown to sporulate in liquid [grown] growth culture. In some media, it also fragments into single cells when the cultures reach the end of vegetative growth. Because the production of most secondary metabolites, including bioactive small molecules, occurs at the end of log growth, it is possible to screen for *Streptomyces venezuelae* fragmented cells that are producing [bioactives] bioactivities by a fluorescence analyzer, such as a FACS machine, given the natural fluorescence of some small molecules.

Applicants: Short and Keller
Application No.: 09/848,095
Filed: May 3, 2001
Exhibit A: Page 5

Please amend Paragraph [0194] on page 57 to read as follows:

(Amended) In the method of the present invention, the fluorescing properties of this and other similar compounds [can] are utilized to screen for compounds of interest, as described previously, or are utilized to enrich for the presence of compounds of interest. Host cells expressing recombinant clones potentially encoding gene pathways are screened for fluorescing properties. Thus, cells producing fluorescent proteins or metabolites can be identified. Pathway clones expressed in [E.coli] *E. coli* or other host cells, can yield bioactive compounds or "backbone structures" to bioactive compounds (which can subsequently be "decorated" in other host cells, for example, in metabolically rich organisms). The "backbone structure" is the fundamental structure that defines a particular class of small molecules. For example, a polyketide backbone will differ from that of a lactone, a glycoside or a peptide antibiotic. Within each class, variants are produced by the addition or subtraction of side groups or by rearrangement of ring structures ("decoration" or "decorated"). Ring structures present in aromatic bioactive compounds are known in some instance to yield a fluorescent signal, which can be utilized to distinguish these cells from the population. Certain of these structures can also provide absorbance characteristics which differ from the background absorbance of a non-recombinant host cell, and thus can allow one to distinguish these cells from the population, as well. Recombinant cells potentially producing bioactive compounds or "backbone" structures can be identified and separated from a population of cells, thus enriching the population for desirable cells. Thus, the method of the present invention also facilitates the discovery of novel aromatic compounds encoded by gene pathways, for example, encoded by polyketide genes, directly from environmental or other samples.

Applicants: Short and Keller
Application No.: 09/848,095
Filed: May 3, 2001
Exhibit A: Page 6

In the claims

Please amend claims 1, 2, 5, 8, 10, 15, 19, 20, 26, 27, 28, 31, 34, 36, 41, and 46 as follows:

1. (Amended) A method for identifying bioactivities or biomolecules using high throughput screening of nucleic acid comprising:

a) providing a gene library containing a plurality of clones, wherein [the] DNA for generating the library is obtained from more than one organism;

b) encapsulating a bioactive substrate and at least one clone of the library in a gel microdroplet, wherein a bioactivity or biomolecule produced by the clone is detectable by a difference in the substrate prior to contacting with the at least one clone as compared to after the contacting; and

c) screening the [clones] microdroplets with an assay or an analyzer that detects [a bioactivity or a biomolecule] the presence therein of the change in the substrate, wherein the change indicates the identity of the bioactivity or biomolecule[; and

d) identifying clones detected as positive for a change in the substrate identifying clones detected as positive for a change in the substrate, wherein a change in the substrate is indicative of DNA that encodes a bioactivity or biomolecule].

2. (Amended) The method of claim 1, wherein the bioactivity is provided by an enzyme that is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases,

lignin peroxidases, diarylpropane peroxidases, epozide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

5. (Amended) The method of claim 4, wherein the *Streptomyces* sp. is *Streptomyces venezuelae*.

8. (Amended) The method of claim [5] 7, wherein the expression library contains DNA obtained from extremophiles.

10. (Amended) The method of claim 9, wherein the [extremeophiles] extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.

15. (Amended) The method of claim 14, wherein the heating occurs [at] for about 30 minutes.

19. (Amended) The method of claim [4] 3, wherein the prokaryotic cell is *E. coli*.

20. (Amended) The method of claim 19, wherein prior to step b), the library in *E. coli* is transferred to a *Streptomyces* sp.

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26. (Amended) A method for identifying bioactivities or biomolecules using high throughput screening of nucleic acid comprising:

a) providing a gene library containing a plurality of clones, wherein the nucleic acid for generating the library is obtained from more than one organism;

b) inserting a bioactive substrate into the clones of the library, wherein a change in the substrate is detectable in the presence of a bioactivity or biomolecule; and

c) screening the clones with an assay or an analyzer that detects [a bioactivity or a biomolecule] the presence therein of the change in the substrate, wherein the change in the substrate identifies the bioactivity or biomolecule [; and

d) identifying clones detected as positive for a change in the substrate identifying clones detected as positive for a change in the substrate, wherein a change in the substrate is indicative of DNA that encodes a bioactivity or biomolecule].

27. (Amended) The method of claim 26, further comprising [encapsulation] encapsulating the clone and the bioactive substrate prior to screening.

28. (Amended) The method of claim 27, wherein the bioactive substrate is a polynucleotide encoding an enzymatic substrate and the bioactivity is provided by an enzyme that is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epozide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

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31. (Amended) The method of claim 30, wherein the *Streptomyces* sp. is *Streptomyces venezuelae*.

34. (Amended) The method of claim [31] 33, wherein the expression library contains DNA obtained from extremophiles.

36. (Amended) The method of claim 35, wherein the [extremeophiles] extremophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.

41. (Amended) The method of claim 40, wherein the heating occurs [at] for about 30 minutes.

Please add the following new claims 54-55:

--54. (New) The method of claim 26, wherein the bioactive substrate is a polynucleotide encoding a fusion protein comprising the substrate flanked by two fluorescent proteins that upon contact cause a change in fluorescent signal from the clone, and wherein the effect of the presence of the biomolecule or bioactivity is to cause such contact.

55. (New) The method of claim 54, wherein the substrate is a thioesterase.--